6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring *N*-nitrosodiphenylamine in environmental media and in biological samples. The intent is not to provide an exhaustive list of analytical methods that could be used to detect and quantify *N*-nitrosodiphenylamine. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used to detect *N*-nitrosodiphenylamine in environmental samples are the methods approved by federal organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are. included that refine previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL MATERIALS

Analyrical methods exist for measuring *N*-nitrosodiphenylamine and its metabolites in the serum, blood, and urine of animals. However, because the data are very limited, a comparison of the methods cannot he made. The methods used are differential pulse polarography (DPP), thermal energy analyzer (TEA) (Pylypiw and Harrington 1981), high-performance liquid chromatography (HPLC) combined with detection by ultraviolet (UV) or UV-visible (UV-VIS) detection, or gas chromatography (GC) with detection by mass spectrometry (MS) (Appel et al. 1984; Tatsumi et al. 1983). Preparation steps for DPP and TEA include the addition of a buffer to the sample, which is then passed-through a Se*p*-Pac® cartridge. The cartridge is washed with methanol (for DPP) or methylene chloride [for TEA), diluted with a buffer, concentrated under nitrogen (for TEA), and analyzed by DPP or TEA. Results were included only for *N*-nitroso-*N*-methylaniline. The authors stated that results for *N*-nitrosodiphenylamine were comparable (Pylypiw and Harrington 1981). Detection limits obtained using DPP analysis for blood, serum, and urine were 0.5, 0.05, and 0.1 ppm, respectively. Recovery was good for all sample types, ranging from 85% to 101%; precision was excellent (<5%). Better sensitivity was obtained using TEA rather than the DPP. Detection limits obtained using TEA analysis for blood and serum were both 0.01 ppm. Recovery was good for both sample types, ranging from 82% to 96%; precision was excellent (<3%) (Pylypiw and Harrington 1981). No information was given on sensitivity, precision, or recovery using HPLC/UV, HPLC/UV-VIS, or GC/MS (Appel et al. 1984; Tatsumi et al. 1983). Table 6-1 gives a summary of the methods available for analyzing for *N*-nitrosodiphenylamine in biological materials.

6.2 ENVIRONMENTAL SAMPLES

Methods exist for measuring *N*-nitrosodiphenylamine in air, water, soil, and foods. The most common methods used are GC and HPLC combined with detection by TEA, flame-ionization detector (FID), or MS. Methods for analyzing for *N*-nitrosodiphenylamine in environmental samples are summarized in Table 6-2.

N-Nitrosodiphenylamine is measured in air samples using GC/TEA and HPLC/TEA (NIOSH 1983). Air samples are collected with a ThermoSorb®/N air sampling cartridge designed specifically for collection of airborne *N*-nitrosamines. A polar solvent (acetone, methanol, or methanol/dichloromethane) is then backflushed through the cartridge. The eluate is examined using GC/TEA and HPLC/TEA. The TEA has been specifically designed for the detection of *N*-nitroso compounds in the ppb range. TEA is not totally specific for *N*-nitroso compounds. Additional confirmation of positive results can be obtained by the use of HPLC/TEA. Specific sensitivity, recovery, and precision data were not reported. A group in Czechoslovakia reported 100% recovery of *N*-nitrosodiphenylamine from air samples using a silica gel

TABLE 6-1. Analytical Methods for Determining *N*-Nitrosodiphenylamine in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood, serum, urine	A buffered solution containing the sample is passed through a Sep-Pac® cartridge. The cartridge is washed with methanol or methylene chloride, concentrated under nitrogen (for TEA).	DPP	0.5 ppm (blood), 0.05 ppm (serum), 0.1 ppm (urine)	93–99 (blood), 85–90 (serum)	Pylypiw and Harrington 1981[a]
		TEA	0.01 ppm (blood and serum)	82–84 (blood), 88–90 (serum)	
Blood (*N*-nitrosodiphenylamine and metabolites)	Blood sample centrifuged; serum extraction.	HPLC/UV GC/MS	NR NR	NR NR	Tatsumi et al. 1983
Urine (metabolites)	Extracted with dichloromethane; concentrated.	HPLC/UV-VIS	NR	NR	Appel et al. 1984

[a]Actual data are for *N*-nitroso-*N*-methylaniline. Authors indicate that *N*-nitrosodiphenylamine yielded comparable results.

DPP = differential pulse polarography; GC = gas chromatography; HPLC = high-performance liquid chromatography; MS = mass spectrometry; NR = not reported; TEA = thermal energy analysis; UV = ultraviolet detection; UV-VIS = ultraviolet-visible detection

TABLE 6-2. Analytical Methods for Determining *N*-Nitrosodiphenylamine in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Samples collected with ThermoSorb®/N air sampling cartridge. Extraction with acetone, methanol, or methanol/dichloromethane.	GC/TEA HPLC/TEA	NR	NR	Fajen et al. 1979, 1980; NIOSH 1983
Waste water	Extraction with methylene chloride, column clean-up with Alumina®; dry over sodium sulfate; concentrate; solvent exchange to methanol.	GC/TEA GC/FID	NR	86 (TEA) 98 (FID)	Rhoades et al. 1980
Water	Extraction with methylene choride, column clean-up, dry over sodium sulfate; concentrate; solvent exchange to methanol.	GC/MS HRGC/MS		68 (GC/MS) 89 (HRGC/MS)	Eichelberger et al. 1983
Soil	Extraction with appropriate solvent; concentration.	GC/TEA HPLC/TEA	NR	NR	NIOSH 1983
Foods (vodka)	Extraction with dichloro-methane; dry over sodium sulfate; concentrate; redissolve in dichloromethane.	HPLC/TEA	NR	60	Fine et al. 1976

FID = flame ionization detection; GC = gas chromatography; HPLC = high-performance liquid chromatography; HRGC = high-resolution gas chromatography; MS = mass spectrometry; NR = not reported; TEA = thermal energy analysis

column for collecting the sample and eluting with ethanol (Mejstrik et al. 1989). Precision and sensitivity were not reported, and experimental details were limited.

N-Nitrosodiphenylamine is measured in water samples using GC/TEA, HPLCTEA, GC/FID, and highresolution gas chromatography (HRGC)/MS (Eichelberger et al. 1983; NIOSH 1983; Rhoades et al. 1980). Sample preparation steps involve extraction with methylene chloride, column clean-up with Florisil® or Alumina®, drying over sodium sulfate, and concentration in a Kuderna-Danish® evaporator with solvent exchange to methanol. *N*-Nitrosodiphenylamine decomposes thermally in the hot gas chromatograph injection port to nitric oxide and diphenylamine and is measured as diphenylamine. Therefore, this compound cannot be accurately measured in a sample unless it is first separated from diphenylamine prior to GC. Florisile®or Alumina® column clean-up is usually employed for this separation (Eichelberger et al. 1983; Rhoades et al. 1980). Average recovery using GCTEA without Alumina® column clean-up was 86% while average recovery using GC/FID with Alumina® column clean-up was 98%. Sensitivity was not reported (Rhoades et al. 1980). Recovery using GC/MS was 68%. Greater accuracy was obtained using HRGCMS (89%). Precision for both of these methods was adequate (13-14%). Sensitivity was not reported (Eichelberger et al. 1983).

N-Nitrosodiphenylamine can be measured in soil using GC/TEA and HPLC/TEA (NIOSH 1983). Sample preparation involves extraction with an appropriate solvent and concentration. Precision, accuracy, and sensitivity for these methods were not reported.

N-Nitrosodiphenylamine has been measured in foods using HPLC/TEA (Fine et al. 1976). Preparation steps for liquor samples involve extraction with dichloromethane, drying over sodium sulfate, concentration under a vacuum, and redissolving in dichloromethane. Recovery for this method is fair (60%). Sensitivity and precision were not reported.

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of *N*-nitrosodiphenylamine is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of *N*-nitrosodiphenylamine.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA They are defined as substance-specific informational needs that if met would reduce or eliminate the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. No human studies were located regarding methods for determining levels of the compound in blood, serum, or urine. Animal data on the determination of *N*-nitrosodiphenylamine and its metabolites in these media are very limited (Appel et al. 1984; Pylypiw and Harrington 1981; Tatsumi et al. 1983). Sensitivity is in the ppm range (Pylypiw and

Harrington 1981). More information on the sensitivity, accuracy, and precision obtained for these methods is needed to evaluate the value of using levels of *N*-nitrosodiphenylamine as an indicator of exposure. The lack of human data for evaluation of these methods makes it difficult to assess whether these methods are sensitive for measuring background levels in the population and levels at which health effects might occur.

Currently, no hiomarkers of effect have been identified for *N*-nitrosodiphenylamine.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Data on the methods used for determining *N*-nitrosodiphenylamine in air (Mejstrik et al. 1989), water (Eichelberger et al. 1983; NIOSH 1983; Rhoades et al. 1983, soil (NIOSH 1983*),* and foods (Fine et al. 1976) are very limited. More information on the accuracy, precision, and sensitivity for these methods is needed to determine if these methods are sensitive enough to measure background levels in the environment, as well as levels at which health effects might occur. Research investigating the relationship between levels measured in air, water, soil, and foods and observed health effects could increase our confidence in existing methods and/or indicate where improvements are needed.

6.3.2 On-going Studies

No on-going studies were located for *N*-nitrosodiphenylamine.